REMARKS

Status of the Claims

Claims 1-4, 8-20, 39-42, 44, and 47 were previously cancelled.

Claims 21-23 and 30-38 stand withdrawn pursuant to the restriction requirement.

It is respectfully requested that the Examiner reconsider the withdrawal of claim 31. This request was put forth in the previous amendment, but was not addressed in the Office Action. It is requested that this request be considered at this time. Claims 30-38 were withdrawn as being directed to a method, rather than to the antibody fragments. Claim 31, however, is directed to the fragments, not the method. Claim 31, which previously depended from claims 1 and 24, was amended in the last response to depend only from claim 24, currently under consideration. It is respectfully submitted that it is proper to consider the patentability of claim 31 at this time. Claim 31 also is amended herein for clarity; no change in the scope of claim 31 is intended by this amendment.

Applicant expressly preserves the right to pursue any of the withdrawn and cancelled claims in a subsequent continuation or divisional application.

The allowance of claims 5-7 is noted with appreciation.

Other claims currently under consideration are claims 24-29, 43, 45-46, and 48-56.

New claims 57-60 depend directly or indirectly from amended claim 26.

New claim 61 relates to subject matter previously presented in claim 4, now cancelled.

The withdrawal of various previous rejections and objections as noted in pages 2-3 of the Office Action is noted with appreciation.

Claim Objections

Claim 26 is objected to as failing to add another limitation to claim 24 from which it depends. Without acquiescing in the basis of the objection, claim 26 is amended herein to be in independent form, thereby rendering this objection moot. New claims 57-60 which depend from claim 26 correspond to claims 27-29 and claim 25, which depend from claim 24.

Claims 45-46 and 53-54 are objected to as encompassing a molecular weight of the PEG effector molecule beyond the range recited in base claims 24 and 48 from which they depend. Accordingly, claims 24 and 48 have been amended to delete the average molecular weight limitations therein, and claims 45, 46, 53, and 54 have been amended to recite the effector molecule molecular weights disclosed at page 8, lines 10-12 of the specification as originally filed. It is respectfully submitted that this amendment is sufficient to overcome this objection.

Claim rejections – 35 U.S.C. § 112, second paragraph

Claims 24-29, 43, and 45-46 were rejected under 35 USC 112 first paragraph on the grounds that the recited effector molecule molecular weight ranges added new matter to the application. It is respectfully submitted that the amendments to the claims with respect to these limitations, as discussed above in the context of the claim objections, also are sufficient to overcome this ground of rejection.

Claims 24-29, 43, and 45-46 also stand rejected under 35 USC 112 first paragraph on the ground that the limitation "at least two effector molecules" added new matter to the application. The Examiner is respectfully referred to the recitation of "two or more effector molecules" in the Abstract at line 2 and in the specification as originally filed at least at page 1, line 2; page 3, lines 3-4 and 12; page 10, lines 21-22 and 24-25; and page 11, line 19. The claims have been amended to replace "at least two" with "two or more," so that the language of the claims will exactly correspond to the language of the specification. No change in the scope of the claims is intended by this amendment. It is respectfully submitted that this amendment is sufficient to overcome this ground of rejection.

Claim rejections – 35 U.S.C. § 103

Reference is made to the Declaration of David P. Humphreys filed herewith.

The present invention relates, *inter alia*, to antibody Fab and Fab' fragments wherein the heavy chain and light chain are not covalently linked, the fragments having

two or more effector molecules attached thereto, and wherein at least one of the effector molecules is attached to a cysteine in the light chain or heavy chain constant region (specification, p. 3, lines 1-14). The claimed invention is based on the surprising discovery that Fab and Fab' fragments having no covalent bond between the light and heavy chains, and having two or more effector molecules attached thereto, at least one of which is attached to either the heavy or light chain, can exhibit antigen affinity and *in vivo* and *in vitro* stability comparable to wild-type antibody fragments (specification at page 3, lines 5-7).

The present application arose from the search for a more efficient way of providing two or more effector molecules such as PEG on a Fab or Fab' fragment. At the time of the present invention, it was known that PEGylation of antibodies and antibody fragments could improve *in vivo* stability. It also was known that site-selective attachment of effector molecules could be achieved by attaching the effector molecules to cysteine residues in the fragment. It was known that in order to attach an effector molecule, the cysteine would have to be reduced to a thiol by subjecting the molecule to a reduction step. At the time of the invention, it was common wisdom in the art that the interchain disulfide bond between the heavy and light chains had to remain intact during this reduction step in order to preserve the antigen-binding affinity of the fragment. It was believed that if the interchain bond were absent, then the light chain would disassociate from the heavy chain, particularly once an effector molecule was attached. Therefore, reduction reactions were carried out under mild conditions, so that the interchain bond cysteines would not be reduced. These mild reactions conditions resulted in inefficient PEGylation reactions. (Humphreys ¶3)

Prior to the present invention, mutations to create Fab' antibody fragments lacking inter-chain (LC-HC) bonds were known, but these Fab' fragments had not been PEGylated. Specifically, Rodrigues et al (1993) and WO 99/15549 taught that Fab' lacking inter-chain disulfide bonds could be expressed and purified using normal methods. Both of these references are directed to improving the efficiency of $F(ab')_2$ formation *in vitro* from Fab' fragments. Neither Rodriguez et al. (1993) nor WO 99/15549 taught or suggested PEGylation of those Fab' fragments, although WO 99/15549 taught PEGylation of certain species of di-Fab'. Neither of these works taught

or suggested that Fab' lacking inter-chain disulfide bonds might be suitable for *in vivo* applications. (Humphreys ¶4) Other references from that time period demonstrate the then-prevailing belief in the importance of maintaining the interchain disulfide bond. In particular, it was known that certain light chain dimers were associated with particular diseases, which indicated that light chain loss or exchange from a Fab'-PEG molecule with no interchain disulfide bond was a risk. (Humphreys ¶¶5, 6)

Fab' fragments that had been site-specifically mono-PEGylated at the hinge were known, but only those with intact inter-chain (LC-HC) bonds (cf. Chapman et al. (1999)). This is consistent with the general understanding in the art at that time that the inter-chain bond had to be intact in order to maintain good binding affinity to antigen. This reference is also consistent with the then-prevailing view that effector molecules were most desirably attached at the hinge portion of the fragment in order to prevent interfering with the antigen binding function of the fragment. (Humphreys ¶7)

The present invention relates to Fab' and Fab antibody fragments lacking inter-chain (LC-HC) bonds and having two or more effector molecules attached, at least one of the effector molecules being attached at either the light chain or the heavy chain of the fragment. At the time of the present invention, little was known about how to predict the stability *in vivo* of Fab and Fab' molecules with altered structures. It was known that 'unstable' molecules such as scFv and human IgG4 were prone to 'domain exchange' both *in vitro* and *in vivo*. Since the long serum permanence was conveyed by the PEG molecule which was covalently attached to the heavy chain, it seemed highly plausible that in Fab'-PEG lacking inter-chain (LC-HC) bonds the light chain might be exchanged or lost in the circulation. This would have resulted in loss of antigen binding function (loss of efficacy) or increased clearance of the protein through precipitation, aggregation or proteolysis. (Humphreys¶9)

In the present invention, either the light chain or the heavy chain of the Fab or Fab' fragments is modified by replacing one of the cysteines that had formed the inter-chain disulfide bond with another amino acid, thereby destroying the interchain bond, and ensuring that the site of cysteine replacement could not subsequently become a site for attachment of an effector molecule. As preservation of the interchain disulfide bond was no longer a criterion, it was possible to use stronger reaction conditions during the

reduction of the cysteines to thiols, allowing for more efficient multiple PEGylation; in fact, as reported in Example 1 of the specification, the inventors herein were able to achieve >65% multiple PEGylation with either two or three PEG molecules using TCEP reductant. Surprisingly, the modified Fab'antibody fragments with 2 or 3 PEG molecules and having no covalent bond between the light and heavy chains were active and stable in the circulation of mice for over 140 hours. Further, there was no loss of antigen binding affinity. This result was wholly unexpected, because the literature at the time suggested that the absence of an interchain bond would result in instability of the fragment in terms of loss or exchange of the light chain in vivo. It had been believed that this instability could be even greater if a large PEG effector molecule was bound to the light or heavy chain. (Humphreys¶10)

Independent claim 24 recites the embodiment wherein the heavy chain in the fragment is not covalently bonded to the light chain, an effector molecule is attached to an interchain cysteine of C_L, and an interchain cysteine of C_H1 has been replaced by another amino acid. Independent claim 26 as amended herein recites the embodiment wherein the heavy chain in the fragment is not covalently bonded to the light chain, the fragment comprising two or more effector molecules, wherein one effector molecule is attached to a cysteine in the light chain constant region and one effector molecule is attached to a cysteine in the heavy chain constant region, and the two cysteines would otherwise be linked to each other via a disulfide bond if the effector molecules were not attached.

Claim 48 recites the embodiment wherein the heavy chain in the fragment is not covalently bonded to the light chain, an effector molecule is attached to an interchain cysteine of C_H1, and an interchain cysteine of C_L has been replaced by another amino acid. Claims 49-54 depend directly or indirectly from claim 48, and correspond to claims 27-29, 43, and 45-46, each of which depends directly or indirectly from claim 24. Independent claims 55 and 56 are similar to claims 24 and 48 respectively, and further recite the presence of the hinge and that any additional effector molecules are attached to the hinge.

New claims 57-61 are described above.

Response to Rejections

The rejection of claims 24-25, 27-29, 43, 45-46 and 48-56 as obvious over Chapman in view of hsei and Humphreys (WO 99/15549) is respectfully traversed.

The combination of the Chapman, Hsei, and Humphreys references does not teach or suggest the present invention. The disclosures of each of these references will be discussed first singly, then in combination.

(a) The Chapman reference

Chapman et al. discloses that Fab' fragments having *intact* inter-chain disulfide bonds and having a *single* PEG attached at the *hinge* region have improved half-lives without loss of antigen-binding affinity. This is consistent with the general understanding in the art at that time that the inter-chain bond had to be intact in order to maintain good binding affinity to antigen. (Humphreys ¶6) Chapman does not teach or suggest that any modification should be made to either the light chain or the heavy chain of the Fab' fragment. Fig. 2 of the Chapman et al. disclosure specifically shows an intact covalent bond between the light and heavy chains, thus teaching away from the presently claimed invention. It is true that Chapman teaches site-specific attachment of PEG molecules, but Chapman teaches that all effector molecules should be attached to the hinge, not to one of the chains as is presently claimed. To one skilled in the art, this reference suggests that in order to have stability in vivo, an interchain bond is necessary. (Humphreys ¶11)

(b) The Hsei reference

Hsei et al. does not teach Fab or Fab' fragments having two or more PEG effector molecules and wherein either the cysteine of C_H1 has been replaced by another amino acid and the cysteine of the C_L is PEGylated, or the cysteine of C_L has been replaced by another amino acid and the cysteine of the C_H1 is PEGylated, or wherein both the cysteine of C_H1 and the cysteine of C_L are PEGylated. On the contrary, where the fragment is Fab or Fab', and a polymer molecule is conjugated to a cysteine residue in the light or heavy chain, Hsei is very careful to say that the entire conjugate can have *only one polymer molecule* attached.

The Hsei reference suggests PEGylation of various antibody fragments, but does not teach any Fab or Fab' fragment having more than one polymer molecule attached wherein one of the molecules is attached at a site other than the hinge. Hsei teaches that a polymer molecule can be coupled to the light or heavy chain of a Fab of Fab' only when there is only one polymer molecule attached to the fragment. Hsei teaches nothing about modification of the interchain disulfide bond when the PEGylation takes place at the hinge. Hsei teaches that one of the interchain cysteines is to be replaced with a serine only when there is only one effector molecule attached to the fragment. (Humphreys ¶15)

Hsei describes but does not exemplify site specific attachment of a PEG molecule to any cysteine other than a single cysteine in the hinge. Hsei describes but does not exemplify attachment of two PEG molecules to Fab or Fab'. Hsei describes and exemplifies attachment of 2 PEG molecules to $F(ab')_2$ molecules, but only by using random amine (lysine) specific NHS-PEG chemistries followed by chromatographic separation of unPEGylated, monoPEGylated and diPEGylated $F(ab')_2$ fragments. Said $F(ab')_2$ molecules were formed from Fab' molecules with a C-terminal leucine zipper dimerisation motif followed by proteolytic cleavage and removal of the leucine zipper. Hsei neither describes nor exemplified methods for the reduction / activation of interchain cysteines for PEGylation without disruption of the inter-hinge disulfide bonds which stabilize the dimeric $F(ab')_2$ structure. (Humphreys ¶16)

The approach taken by Hsei is clearly differentiated from the approach taken in the present application, which enables specific control over both the site and number of PEG molecules attached, namely, attachment of two or more PEG molecules to a Fab or Fab' fragment, and wherein at least one of the PEG molecules is attached to a cysteine in the heavy or light chain constant region. In addition the present application describes precise methods for very efficient PEGylation reactions. Since Hsei did not make Fab' variants containing disrupted interchain disulfide bonds, it was not possible to foresee that one such variant forms very effectively an interchain disulfide bond between the C-terminal cKappa cysteine and the single hinge cysteine (see lane 4 figure 3b), nor then that such a disulfide bond would retain very significant Fab' thermal stability (see pDPH225 in Table 1 of our application), thereby allowing for highly efficient PEGylation

at those two sites. Hsei does not teach any practicable or efficient method of PEGylating such molecules. The present application shows the surprising result that even 5mM DTT compared to the 0.2mM DTT described in Hsei is ineffective at reducing any such 'unexpected' disulfide bonds to the extent required for high levels of PEGylation (application Figure 1). Furthermore, the present application provides evidence that stronger reducing conditions using different classes of reducing reagents (such as TCEP) are required in order to result in efficient PEGylation of such disulfide engineered Fab and Fab' molecules. It is also clear that 'strong' reducing reagents such as TCEP could not be used to reduce and PEGylate $F(ab')_2$ molecules without reducing them to Fab'. Hsei does not teach or suggest to one skilled in the art that it would be desirable to attach two or more effector molecules to such a Fab or Fab' fragment, and provides no motivation or methods for doing so. (Humphreys ¶17)

At page 28, line 32 – page 30, line 5, Hsei generally discusses conjugates of antibody fragments with more than one PEG molecule, but does not discuss the type of fragments, or where on the fragments the PEG molecules are attached. At page 30, line 6 – page 31, line 24, Hsei discusses Fab, Fab', Fab'-SH and $F(ab')_2$ fragments with more than one PEG molecule, but does not discuss where the PEG molecules are attached.

The Action states at page 7, "Hesi [sic: Hsei] et al. teach anti-IL-8 Fab, Fab' and Fab-SH fragments for the treatment of inflammatory disorders wherein the antibody fragments may be conjugated to a polyethylene glycol (PEG) molecule via a cysteine residue in the light or heavy chain of the antibody fragment that would ordinarily form the disulfide bridge linking the light and heavy chains wherein the disulfide bridge is avoided by substituting one of the interchain cysteines for another amino acid, such as serine...." The Action fails to note, however, that in the only embodiments of Hsei in which an interchain cysteine is substituted with another amino acid, there is *only one* effector molecule attached to the fragment (Hsei p. 23, lines 9-14). Nor is Hsei enabling for Fab or Fab' fragments containing more than one effector molecule wherein the effector molecules are attached at any location other than the hinge.

The only Fab and Fab' fragments disclosed in Hsei that have more than one polymer molecule attached have *all* of the molecules attached *only* in the *hinge* region.

Thus the Hsei reference actually teaches away from the surprising result obtained by the present invention.

(c) The Humphreys reference

Humphreys WO99/15549 is concerned with the production of dimeric $F(ab')_2$ fragments and is directed to a peptide TCPPCPXYCPPCPA, which, when part of a Fab' fragment, efficiently generates $F(ab')_2$ dimers (p. 2, lines 3-6). The peptide of interest contains *four* cysteines and is disposed in the Fab' *hinge* region; the dimers include two monomeric chains covalently linked through one, two, three, or four of the cysteine residues of the hinge region peptide of each chain (p. 6, lines 27-32). Effector molecules such as PEG can be attached to the dimers, preferably to the cysteine residues in the peptide sequence (p. 8, line 33 – p. 9, line 36), which, as noted, are in the *hinge* region. In Example 1, the reference teaches the production of di-Fab' from Fab' constructs. Before the dimers are formed, the interchain disulfide bonds are removed from the Fab' constructs, and the interchain cysteines are changed to serines (p. 12, lines 23-30). The breaking of bonds and removal of the cysteines was done to minimize any possible incorrect interchain disulfide bonds between hinge regions and any other cysteines (p. 12, lines 25-26). In particular, both cysteines were removed to minimize such incorrect bonds when the two Fab' fragments come together to form the $di(Fab')_2$ dimer. The issue of such incorrect bonds does not arise when the fragments are to be used in the form of un-dimerized Fab'. (Humphreys ¶12)

In the reference, no effector molecules were attached to the Fab' constructs (p. 12, line 23 – p. 19, line 4). The reference states that the presence of extra *hinge* cysteines above the single one found in the original Fab' was the most important factor in promoting di-Fab' formation in vivo (p. 18, lines 10-12), thus emphasizing the importance of the hinge region, not the interchain regions.

Example 2 is directed in part to *hinge specific* PEGylation of $F(ab')_2$ molecules with modified hinges. $F(ab')_2$ is the only fragment type that is disclosed as being PEGylated (p. 19, line 5 – p.24, line 24). The only disulfide bonds of interest are in the hinge regions (p.22, line 32 – p. 23, line 5; Figure 4) (Humphreys ¶13). PEGylation is discussed at p. 24, lines 8-24. Conditions were selected to retain the molecule as a

dimeric species (p. 24, line 11). An assay showed 1.034 ± 0.090 thiols per $F(ab')_2$ being liberated for reaction (p. 24, lines 20-21), and the efficiency of PEGylation was $\leq 1.3\%$ (p. 24, lines 22-24). Thus, the Humphreys reference differs from the present invention in the following ways: i) Humphreys is directed to processes for making di-Fab' molecules, ii) Humphreys only PEGylates di-Fab' molecules, iii) only one PEG is attached per molecule, iv) the PEG is attached only in the hinge region, and v) the efficiency of attachment is $\leq 1.3\%$. Humphreys makes no mention of Fab or Fab' fragments having effector molecules attached, and no mention of any type of fragment having an effector molecule attached to an interchain cysteine. Thus, the Humphreys reference teaches away from the surprising result obtained in the present invention.

(d) <u>The combination of references does not render the presently claimed invention obvious</u>

At pages 7-8, the Action states, "It would have been *prima facie* obvious to one of ordinary skill in the art at the time the claimed invention was made to have produced anti-IL-8 Fab or Fab', fragments comprising a hinge region having one or two cysteines (e.g., SEQ ID Nos:1-3) and wherein one or [sic: of] the interchain cysteines (CH1 or CL) is mutated to a serine and wherein the hinge cysteine(s) and the interchain cysteine (CH1 or CL) are PEGylated...." because

- Chapman teaches "site-specific attachment of PEG molecules ...to Fab' fragments at one or two engineered hinge cysteine residues
- Hsei teach that antibody fragments may be conjugated to a polyethylene glycol (PEG) molecule via a cysteine residue in the light or heavy chain of the antibody fragment that would ordinarily form the disulfide bridge linking the light and heavy chains wherein the disulfide bridge is avoided by substituting one of the interchain cysteines for another amino acid such as serine
- Humphreys teach known Fab' hinge region peptides (Identical to instantly claimed SEQ ID Nos: 1-3) which are well tolerated in E.coli and are non-immunogenic.

But these selected teachings, cherry-picked from the references and taken out of context, fail to consider that

- In Chapman the interchain bond must remain intact, and the area of site-specific attachment is the hinge, and that it is important that the PEG molecules be "situated well away from the antigen-binding region." (Chapman, p. 780, col. 2)
- Hsei teaches that a cysteine residue in the light or heavy chain can be substituted for another amino acid only when no more than one effector molecule is to be attached to the fragment
- The hinge peptides taught in Humphreys have four cysteines so as to better promote di-Fab' formation, and are *not identical* to the hinge peptides taught in the present application which have only one or two cysteines. Moreover, Humphreys teaches at col. 9, lines 65-67 that both of the interchain cysteines are removed, while the present claims recite that at most only one or the other of the interchain cysteines is removed.

The Examiner discusses the teachings of Hsei and Humphreys relative to cysteines that may be engineered into the *hinge* region, but these teachings suggest nothing about the attachment of effector molecules at the cysteines in *the light chain or heavy chain,* as recited in the present claims. Hsei et al. teaches that Fab' fragments can have more than one effector molecule only when the effector molecules are all at the hinge; and Humphreys teaches effector molecules only on $F(ab')_2$ fragments, and then only in the hinge.

One skilled in the art would not combine the Chapman, Hsei, and Humphreys references in the manner suggested by the patent examiner. Specifically these references would not teach one skilled in the art to place effector molecules on both the hinge and the light or heavy chain at the site of the interchain cysteine bond, as suggested by the Examiner. There is no reason for one skilled in the art to combine these references. Moreover, the prevailing view in the art that destroying the interchain bond would diminish antigen binding affinity would lead one skilled in the art away from removal of both interchain cysteines. (Humphreys ¶18)

Furthermore, the data presented in the specification demonstrate the unexpected results achieved with the inventive antibody fragments. The Examiner is directed to Table 1 and antibodies pDPH225 and pDPH252, which show good levels of purification even at the elevated temperature of 60ºC. See also, page 22, lines 2-7. Table 2 also shows that antibody pDPH225 has good Kd values of 8.5. The Examiner is respectfully reminded that a Kd value is a reciprocal value and thus the lower the lower the value the better. It is unexpected that these antibodies would exhibit such good stability under these conditions.

The present rejection is based on selection of elements from the prior art from among numerous possible choices and combinations without guidance from the art to combine the elements as the applicants have done. Yet "an invention would not have been obvious to try when the inventor would have had to try all possibilities in a field unreduced by direction of the prior art. When 'what would have been obvious to try would have been to "vary all parameters or try each of numerous possible choices until one possibly arrived at a successful result, where the prior art gave either no indication of which parameters were critical or no direction as to which of many possible choices is likely to be successful, and invention would not have been obvious" *Bayer Schering Pharma AG v. Barr Laboratories Inc.*, 91 USPQ2d 1569, 1572-73 (Fed. Cir. 2009). Second, an invention is not obvious to try where vague prior art does not guide an inventor toward a particular solution. A finding of obviousness would not obtain where "what was 'obvious to try' was to explore a new technology or general approach that seemed to be a promising field of experimentation, where the prior art gave only general guidance as to the particular form of the claimed invention or how to achieve it." Id. at 1573.

The Examiner is applying a prohibited hindsight analysis, starting with the applicants' invention and using it as a template from which to extract unrelated teachings of the prior art. Yet the prior art, when taken as a whole, actually teaches away from the present invention. Hsei teaches that more than one effector molecule can be bound to a heavy or light chain only when the fragment is $F(ab')_2$; all other fragments must have either (a) only one effector molecule attached to a heavy or light chain cysteine, or (b)

more than one effector molecule, all of which are attached at the hinge region. This would lead one away from attempting to create an antibody Fab or Fab' fragment with more than one large effector molecule, at least one of which is attached to the heavy or light chain.

As the Examiner correctly notes, the test of obviousness is what the combined teaching of these references would have suggested to those of ordinary skill in the art at the time the invention was made. The preparation of the claimed multi-PEGylated fragments was difficult, as illustrated by Figure 1 of the present application, showing that the inventors had to carefully determine which reductants would give them the desired multi-PEGylation. To the extent that the Examiner notes that some degree of predictability is required for obviousness, the fact that the applicants had to try several reductants and achieved varying degrees of success shows that this is an unpredictable art, further supporting the non-obviousness of the presently claimed invention.

In view of the foregoing, it is respectfully requested that the rejection under 35 U.S.C. 103 be withdrawn.

Double patenting

The double patenting rejection based on claims 7 and 10 of U.S 6,642,356 is respectfully traversed.

The '356 patent is the U.S. equivalent of the Humphreys reference discussed above. The only teaching of the '356 patent with respect to the interchain cysteines is that *both* cysteines need to be removed, and then only so that no incorrect bonds will be formed when the Fab' fragments dimerize to di-Fab'. The focus of the '356 patent disclosure is a peptide sequence that can be used as hinge regions in proteins, where they can be covalently coupled to achieve dimeric structures, for example, as found in antibodies. This hinge protein has nothing to do with the interchain cysteines. Independent claim 5 of the '356 patent recites an antibody fragment comprising one polypeptide chain having the recited amino acid sequence; claim 7 which depends on claim 5 recites that the fragment is a Fab or Fab' fragment, and claim 10 which depends on claim 7 recites the fragment with one or more effector or reporter molecules attached to it.

Claims 7 and 10 of the '356 patent are specifically drawn to a fragment comprising a polypeptide chain in the hinge region with four cysteine residues. The present application claims fragments with only one or two effector molecules in the hinge regions, and hinge region polypeptides with only one or two cysteines. The polypeptide of the '356 patent is not the same as the polypeptides disclosed and claimed in the present application. (Humphreys ¶14)

The '356 patent teaches nothing about binding an effector molecule to a cysteine on a light chain or a heavy chain, or both, and is solely directed to the use of particular peptides in the hinge region to create dimers. In fact, the '356 teaching that both interchain cysteines are to be removed teaches away from the use of such cysteines as effector molecule binding sites, as claimed herein. Thus one skilled in the art seeking to create Fab or Fab' fragments having effector molecules attached to one or more of the interchain cysteine residues would not have looked to Humphreys '356. The present claims are not obvious variants of claims 7 and 10 of '356.

New claim 61

New claim 61 relates to the subject matter covered by claim 4, previously cancelled based on the rejection of inherent anticipation over Carter in view of Bodmer. Upon further consideration, it is respectfully submitted that this subject matter is not inherently anticipated.

Claim 61 relates to an intermediate in which the interchain cysteine of C_H1 of a Fab' fragment has been replaced by another amino acid, and wherein the C_L cysteine is covalently bonded to a cysteine in the hinge region. This fragment served as an intermediate that could be PEGylated to provide the PEGylated fragments of the present invention. The formation of this bond was unexpected by the inventors herein. It also was surprising that this intermediate fragment provided greater stability than when both interchain cysteines were mutated, as shown at Table 1 of the present application (compare pDPH224 and pDPH225). This greater thermal stability enabled precipitation and removal of many E.coli host proteins and Fab' proteolytic fragments by incubation of E.coli periplasmic extract at elevated temperature. Because the light chain cysteine and hinge cysteine were bound to each other and not available for extraneous bonding, the

intermediate fragment also provided greater analytical simplicity, purity, and protein integrity. (Humphreys ¶19).

The Carter reference relates to expression in E.coli of antibody fragments having at least a cysteine present as a free thiol, used for the production of bifunctional $F(ab')_2$ antibodies. In particular, Carter desires to provide Fab' antibody fragments having at least one hinge region cysteine present as a free thiol (Fab'-SH) while obviating the inherent problems in generating Fab'-SH from intact antibodies (p. 5, lines 24-31). Thus Carter discloses a Fv polypeptide containing an immunoglobulin heavy chain Fv region and an immunoglobulin light chain Fv region, said light or heavy chain also comprising an unpaired cysteinyl residue as a free thiol, and recovering the polypeptide under conditions that substantially maintain the cysteinyl residue as the free thiol. (page 6, lines 9-14) Carter also discloses a Fab' antibody polypeptide having at least one hinge region cysteine present as a free thiol (Fab-SH) (p. 7, lines 4-18). (Humphreys ¶20)

The fragments as taught by Carter do not inherently form bonds between the cysteine on the light chain and the hinge cysteine. Because in Carter the cysteine is present as a free thiol and maintained as a free thiol until it can be reacted to form $F(ab')_2$ dimers (page 8, lines 6-35), the hinge cysteine thiol was not available to react with a light chain cysteine thiol. Moreover, since Carter was not interested in adding effector molecules to the fragment, Carter would not have realized that a Fab' fragment with a bond between the light chain cysteine and the hinge cysteine would have conferred any advantage in a subsequent PEGylation step. (Humphreys ¶21)

It is therefore respectfully submitted that the subject matter of claim 61 is allowable over the art of record.

CONCLUSION

As all points of rejection have been overcome, a Notice of Allowance is respectfully requested. The Examiner is invited to contact the applicant's undersigned representative if it is believed that a conference might further the prosecution of this matter.

Respectfully submitted,

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